

A POLY (ADP-RIBOSE) POLYMERASE INHIBITOR AMELIORATES INFLAMMATION ASSOCIATED WITH DIABETIC NEPHROPATHY

Evidence for the key role of the nuclear enzyme poly (ADP-ribose) polymerase (PARP) in chronic diabetic complications in the heart, retina, and peripheral nerves is emerging. The role for PARP in diabetic nephropathy, a devastating complication of diabetes, has not been properly explored. In our previous studies, we have shown that pharmacological inhibition of PARP prevents urinary albuminuria, one of the key manifestations of diabetic kidney disease. The purpose of the current study is to assess the effect of PARP inhibitors on other endpoints of diabetic nephropathy, and, in particular, on variables that characterize renal inflammation. Control and streptozotocin-diabetic rats were treated with or without the PARP inhibitor, 1,5-isoquinolinediol (ISO, 3 mg kg body weight⁻¹ d⁻¹, intraperitoneally). The inhibitor was administered for ten weeks after two initial weeks without treatment. At the end of the study, concentrations of monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor- α (TNF α) were assessed in the kidney cortex samples of experimental groups. The measures of kidney function and, in particular, urinary total protein and TNF α have also been evaluated.

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INTRODUCTION

Growing evidence suggests that inflammation is an important factor in the development of diabetic nephropathy.¹ Monocytes (macrophages) and their adherence to endothelial cells as well as proinflammatory cytokine production promote progression of diabetes-induced renal changes culminating in fibrosis and renal insufficiency.²⁻⁴ Increased kidney expression of monocyte chemoattractant protein (MCP-1) has been reported related to diabetic kidney disease.⁵ Infiltrated macrophages produce lysosomal enzymes, nitric oxide, reactive oxygen species (ROS), and tumor necrosis factor- α (TNF α),^{6,7} all of which have been implicated in the pathogenesis of diabetic nephropathy. Poly (ADP-ribose) polymerase (PARP) inhibitors counteract inflammation⁸ and prevent and alleviate diabetic complications through multiple mechanisms.^{9,10} One of the PARP inhibitors, 1,5- isoquinolinediol (ISO), was also found to be effective against diabetic neuropathy.¹¹ This study investigated the effect of ISO on renal inflammation in diabetic rats.

METHODS

Urine collection

At the end of the 12-week study, rats of four experimental groups [control (C); ISO-treated control (C+I); Diabetic (D); ISO-treated diabetic (D+I), n= 7-10] were placed into separate metabolic cages for 24 hours. One hundred mL water bottles were placed into the inserts on each cage. The rats were given 24 hours to consume as much water as they desired. The water bottles were

checked and refilled every 8 hours throughout the 24 hour period. During this 24 hour period, a 50 mL centrifuge tube was connected to a screen mesh outlet on the bottom of each metabolic cage. Rat urine was collected from these tubes after 24 hours.

Kidney extracts

The rats were euthanized by sedation with CO₂ followed by immediate cervical dislocation (IACUC approved). Their kidneys were rapidly dissected. Kidney cortex samples were homogenized in the lysis buffer (10 mmol/L Tris-HCl buffered solution, pH 7.4, containing 150 mmol/L NaCl, 1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L EDTA, and 0.01% Triton x-100). The samples were centrifuged at 12,000 rpm for 15 minutes and the supernatants were aliquoted and stored at -80°C.

Total protein assay

One mL urine samples were placed in centrifuge tubes and centrifuged at 3,000 rpm for 5 minutes. After centrifugation, the supernatant was collected from the tubes. These samples were then frozen at -20°C. At the time of analysis, the samples were taken out of the freezer, thawed at room temperature, and centrifuged again. The supernatant was used for total protein assay. Measurements were carried out using the Bradford protein assay method (Sigma, Saint Louis, Mo).

Measurements of MCP-1 and TNF α by enzyme-linked immunosorbent assay (ELISA)

To determine MCP-1 concentrations in the kidney and TNF α concen-

trations in kidney and urine, the ELISA kits for MCP-1 (BD Biosciences, San Diego, Calif) and TNF α (BD Biosciences, San Diego, Calif) were used. All samples were analyzed in duplicates. The concentrations of MCP-1 and TNF α in the kidney samples were normalized for the total protein.

Poly (ADP-ribose) immunohistochemistry

For immunohistochemistry, formalin-fixed, paraffin-embedded kidney tissue sections were used. Staining for poly (ADP-ribose) was performed using mouse monoclonal anti-poly (ADP-ribose) antibody (Trevigen, Gaithersburg, Md). The detailed procedure has been described by us previously.¹² Briefly, after deparaffinization and blocking, sections were incubated with the primary antibody (1:100 dilution) for 2 hours. The signal was developed using the DAB Substrate kit (Vector Laboratories, Burlington, Calif).

RESULTS

Urinary 24-hour TNF α excretion in the experimental groups

The D group displayed a higher urinary TNF α excretion than the C group ($P<0.01$). The D+I group showed a significant decrease in TNF α excretion compared with the D group ($P<0.01$). ISO treatment did not affect urinary 24-hour TNF α excretion in the C+I group.

Renal MCP-1 concentrations in the experimental group

The D group had a higher renal MCP-1 concentration than the C group ($P<0.01$). The D+I group showed a significant attenuation of MCP-1 accumulation compared with the D group ($P<0.05$).

The 24-hour urinary protein excretion in the experimental groups

Proteinuria was significantly higher in the D group than in the C group ($P<0.01$). ISO treatment of diabetic rats significantly reduced urinary protein excretion ($P<0.01$) compared with the untreated diabetic group).

Renal TNF α concentrations in the experimental groups

The D group had a significantly higher TNF α concentration compared with the C group ($P<0.01$). ISO treatment reduced renal TNF α concentration in diabetic rats ($P<0.01$). Increased poly (ADP-ribose) immunoreactivity was found in the D group ($P<0.01$). In contrast, poly (ADP-ribose) immunoreactivity was barely detectable in the D+I group.

CONCLUSION

PARP activation plays an important role in early diabetic nephropathy, and in particular, in renal inflammation. The findings support the rationale for development of PARP inhibitors and PARP inhibitor-containing drug combinations for treatment of diabetic nephropathy.

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